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Laboratory Procedure Handout

IMMUNOGLOBULIN M "IgM"

Immunodiffusion "single diffusion" precipitation immunoassay

INTRODUCTION

IgM represents about **8%** to **10%** of the total serum immunoglobulins and is present on **normal serum** at a **concentration** of **approximately 120 mg/dl**.

A. Structure

a) With an S value of **19S**, **IgM** has a **pentameric** structure, consist of **five monomeric units** linked by a **J chain** and by **disulfide bonds** at the **Fc** fragment.
b) IgM is easily **dissociated** by **reducing agents**, forming **five monomeric units** of **7S** IgM.

B. Biological and chemical properties

a) **IgM** is the **first antibody that an immunologically commited B lymphocyte can produce**. It has a half-life of approximately 10 days.

b) **IgM** will **appear** in the **B cell membrane** (followed shortly by IgD) prior to an encounter with its homologous epitope.
c) **IgM** is the **predominant antibody** in the **early (primary) immune response to most antigens**.
d) **IgM** is **the predominant antibody produced** by the **fetus**. An **elevated IgM level in the cord serum of a newborn** (normal level, approximately 10 mg/dl) may indicate that the fetus was **infected before** birth.

e) **IgM** is **the only antibody made to certain carbohydrate antigens, such as the ABO blood group antigens on human erythrocytes**.
f) **IgM** is **the most efficient immunoglobulin at activating complement in lytic reactions.**
g) **IgM** **is not intrinsically opsonic**, **since phagocytic cells do not possess a receptor for the Fc portion of the μ chain**. However, **IgM enhances**

phagocytosis by causing the **deposition of the C3b opsonin onto the surface where the IgM antibody resides**.

h) **Some IgM** is **synthesized** locally in **secretory tissues** (e.g., parotid glands) **Secretory IgM,** like sIgA, **can bind secretory component**.

Diagnostic Significance

High IgM concentrations are to be found in the early phase of infectious diseases (e.g., viral infections such as infectious mononucleosis, acute hepatitis). An isolated large increase in IgM develops in primary biliary cirrhosis. In the case of liver complains (e.g., cirrhosis of liver) the elevation of IgM is usually less pronounced than that of IgG and IgA. In the case of Waldenstrom's macroglobulinemia a large increase in IgM is observed; the remaining immunoglobulins are reduced, thereby giving rise to the symptoms of an antibody deficiency. The various forms of the antibody deficiency syndrome are associated with low concentration of one or more immunoglobulin classes. In the case of autoimmune diseases (e.g., lupus erythematosus, progressive rheumatoid arthritis) IgM is increased in the serum.

PRINCIPLE

Single (one component is **fixed**) radial immunodiffusion is a precipitation reaction, where the antigen is soluble (**in this case the antigen is the immunoglobulin** with the **μ chain "IgM"**). **The antigen-antibody interaction take places** in a **semisolid medium** (e.g., **agarose-gel layer**), "**bands"** of **precipitation will form**.

REAGENTS

Petri dish contains Agarose-gel layer, contains **monospecific antiserum** to **human IgM (u chain)**. These **antisera** are **obtained** by the **immunization** of **rabbits**, **sheep**, or **goats**. The **preservatives** used in the agar are approximately 1g/l **sodium azide**, and **sodium p-ethyl-mercury-mercapto-benzene-sulfonate** (max. 0.1g/l) the used peti dish is supplied by the **NOR-Partigen company**. The kit is supplied with a **control** and **standards** to **be run along with the specimens** . The **assay range**: **0,23----3,48 g/l (IFCC) or 0,32-4,83 g/l (Behring)**.

Stability & Storage

The NOR Partigen used up to the date given on the label when stored in the original unopened pack at +2 to + 8 C. It is imperative to protect t he plate from freezing (e.g., in the vicinity of the deep-freeze compartment of a refrigerator). Once opened, a plate should be used within a maximum of 4 weeks.

SPECIMINS

Plasma or **serum** sample are **as fresh as possible or** have been **stored deep-frozen**.

METHOD

1. **Remove the plastic container, and allow the opened plate to stand for about 5 minutes at room temp for evaporation of any condensed water which may have penetrated into the wells.**

2. **Dispense exactly 5 µl = 0.005ml (volume required per well) [use Hamilton Microtiter syringe, Eppendorf Micropipette, Behring dispenser, or Partigen dispenser] undiluted patient's serum (except if a suspicion of an IgM-paraproteinaemia, the sample should be examined in a dilution from about 1 + 5 to 1 + 20), control , and the standards.**

Procedure A. (Table of calibration values / 1 control serum).

For checking the accuracy of NOR-Partigen IgM, introduce control serum for NOR Partigen into well 1. Wells 2 to 12 are intended for the specimens to be examined.

Procedure B. (Reference curve / 3 standard solutions).

As an alternative to the routine determination in accordance with procedure A the IgM determination may also be effected by plotting a reference curve. Behring supplies three prediluted standard solutions for this purpose. Introduce Ig/C3c standard serum (human) for NOR Partigen (solutions 1, 2, 3) into wells 1 to 3. Wells 4 to 12 are intended for the specimens to be examined. The accuracy of the method may be checked by introducing the control serum for NOR Partigen into one well. After introduction of the specimens allow the plate to stand tightly closed at room temperature.

EVALUATION

Measurement of the diameters

After expiration of a diffusion period of **5 days measure** the **diameters D** of the **precipitates** to **an accuracy of 0.1 mm using a suitable device** such as the measurement template for NOR Partigen, **a scaled magnifying glass against a black background with lateral illumination**, or the Behringwerke Measuring Viewer for immunoanalysis. **In the case of precipitate ring diameters D > 8.0 mm, the result should be rechecked later, to enable a correction to be made where there has been further diffusion**. **In the case of deviations in the precipitate ring diameter of ± 0.4 mm or more, the error for the result is of a magnitude greater than ± 15 %. The absolute error** is **greater** in the **lower assay range**, **precipitin ring diameters D < 5.5 mm**, **than in the upper range**.

Evaluation after attainment of the diffusion end-point

Procedure A

The corresponding assay results may be ascertained by reading the values from the appended Table of Calibration Values for the precipitate ring diameters measured. The accuracy of these results is checked by means of Control serum for NOR Partingen; in this connectionthe batch-dependent precipitate ring diameter given in the Table of Assigned Value must be confirmed within the confidence range (D = ± 0.3 mm). Confirmation of the assigned values for the control serum for NOR Partigen also guarantees the accuracy of the assay results for the specimens examined.

Procedure B

The squares of the diameters of the precipitates from the standard solutions (wells 1 to 3) are plotted on linear millimeter graph paper as a function of the standard concentrations;

Abscissa: antigen concentration in g/l (Behring) or g/l (IFCC)
Ordinate: squares of the ring diameters in mm

The result is a straight line whose intersection with the ordinate should lie between 8.5 and 13.5 mm^2 . The IgM concentrations corresponding to the precipitate diameters of the patient sera are ascertained from this reference curve.

Evaluation after 18 hours diffusion

Procedure A

At IgM concentration up to about 0.9 g/l (D = 5.0 mm) the diffusion end-point is already attained after 18 hours. With diameters D> 5.0 mm an enlargement of the precipitate is to be expected at a later reading. The readings obtained after 18 hours have the following inferential value for an early diagnosis:

---- concentrations 20 to 60 % of the normal = hypoproteinaemia
---- concentration > 60 % of the normal = normal finding or hyperproteinaemia

The exact results in the hyperproteinemic range can be ascertained after the diffusion end-point has been reached.

Procedure B

The diameters of the 3 standard solutions are plotted on semilogarithmic millimeter graph paper as a function of the standard concentration:

Abscissa: log antigen concentration in g/l (Behring) or g/l (IFCC)

Ordinate: ring diameter in mm

The result is a straight line which permits a relatively exact early evaluation. In the case of an IgM-paraproteinaemia (monoclonal IgM) the IgM determined can differ from the results with other methods because of a possible difference form the physicochemical and immunochemical properties of the polyclonal IgM.

Note

When reference curves are prepared using Ig/C3c Standard serum (human) for NOR Partigen some batches of the standard solutions may additionally develop weak inner rings in the NOR Partigen IgM immunodiffusion plates. This has no effect on the accuracy of the results on plasma/serum samples.

Reference values

	Mean value			Range of variation		
	g / l (IFCC)	g / l (Behring)	% of the normal	g / l (IFCC)	g / l (Behring)	% of the normal
IgM content in the serum of healthy central European men (15 to 64 years old)	0.9	1.25	78	0.432-1.8	0.60-2.50	37-156
Women (15 to 64 year Old)	1.15	1.60	100	0.504 - 2.02	0.70-2.80	43 -175
Children (1 to 1.5 years old)	0.742	1.03	65	± 0.533	± 0.74	46
Neonates (2 to 8 days old)	0.108	0.15	9.4	± 0.072	± 0.10	± 6.3

References

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